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Goat semen preserved at 4 °C until 76 hours before artificial insemination: Different attempts to maintain the fertility

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Abstract

The aim of this study was to evaluate the extender composition, presence or absence of seminal plasma and dilution rate of spermatozoa for the preservation of fresh semen during three days before artificial insemination (AI). The percentage of kidding was influenced by the duration of storage: 4 h = 71%; 28 h = 61%; 52 h = 39%; 76 h = 28%. The fertility rate decreased in both extenders tested (milk or NPPC) when the storage duration increased. Evidence was given that the removal of seminal plasma by centrifugation at collection does not improve the fertility rate. Using the same number of total spermatozoa per straw (100 x 10^6), but with a variable inseminated volume of 0.20 mL or 0.40 mL per straw, AI results showed that the reduction of the concentration of spermatozoa in the AI dose did not improve the fertility rate after AI, whatever the storage duration.

Keywords: Goat, artificial insemination, fresh semen
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Introduction

Artificial Insemination (AI) of dairy goats with fresh semen was initially developed using milk as extender and 4 °C as storage temperature. The fertility rate of the semen generally decreased dramatically after 12 to 24 hours of storage. The objective of this experiment was to optimize the conditions for the preservation of fresh semen to improve the fertilizing capacity of sperm stored until three days before AI. The factors studied were extender composition, presence or absence of seminal plasma and dilution rate.

Materials and Methods

Two experiments were conducted to evaluate the fertility of fresh semen stored before AI up to 76 hours at 4 °C in milk extender or in NPPC-BL extender. This experiment was conducted during the non-breeding season (May to July). Does were synchronised with progestagen-impregnated intravaginal sponges (Chronogest^R ; Intervet, France) for 11 days The does were also treated with 50 µg i.m. of cloprostenol (Hoechst, France), eCG respectively 400 IU (<3.5 kg milk/day) and 500 IU (>3.4 kg milk/day) 48 hours before removal of the sponges. Each doe was inseminated once (exocervical method) at 43rd hours after sponge removal with a straw containing 100 x 10^6 of total chilled sperm (Corteel *et al.*, 1988). In each experiment, goats were randomised into four groups according to the duration of storage semen preservation: 4, 28, 52 or 76 hours. Individual ejaculates were used from nine Alpine and three Saanen adult bucks under photoperiodic treatment for the elimination of seasonal sexual variations (Delgadillo *et al*., 1992). Semen was collected with the aid of an artificial vagina at 37 °C. The experimental extender consisted of native phosphocaseinate and Betalactoglobulin (NPPC-BL) (Leboeuf *et al.*, 2003) diluted in a BesKOH saline solution (Dunner *et al*., 1993) supplemented with glucose (0.01 M). The NPPC-BL originating from cow milk was prepared by microfiltration, diafiltration and freeze-drying (Faulquant *et al*., 1992). Heated skim milk was used as a control (Corteel, 1974). The diluted sperm was packed at room temperature in 0.25 mL or 0.50 mL straws (IMV, L'Aigle, France) and then chilled to 4 °C for 90 minutes.

Experiment 1 was designed to evaluate the effect of seminal plasma (removal: SP-; not removal: SP+) at collection on the fertility (n = 360 goats). Each of 19 ejaculates from eight bucks was split at collection. The experiment was conducted in a 2 x 2 factorial treatment arrangement (SP- *vs.* SP+; NPPC-BL *vs.* milk). The washed technique as described by Corteel (1974) was used.

Experiment 2 was designed to evaluate the effect of dilution rate on the fertility (n = 320 goats). Seminal plasma was separated at collection in each of 21 ejaculates from nine bucks. The experiment was

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conducted as a 2 x 2 factorial treatment arrangement (500 x 10^6 *vs.* 250 x 10^6 total spermatozoa/mL; NPPC-BL *vs.* milk). The separated ejaculates were conditioned in 0.25 mL straws (0.20 mL usable; 100 x 10^6 total sperm) or 0.50 mL straws (0.40 mL usable; 100 x 10^6 total sperm). Sperm characteristics of each ejaculate were assessed immediately after chilling to 4 °C, then after 4 h, 28 h, 52 h and 76 h of storage. Parturition was observed for all inseminated goats and number and sex of kids were recorded. Fertility was determined as % of kidding. The Genmod procedure was used to test treatments of semen and storage duration on kidding rate. The GLM procedure was used to test prolificacy (SAS, 1990).

Results and Discussion

The percentage of kidding was influenced by the duration of storage (P = 0.001). In Experiment 1 the fertility of the semen decreased progressively with increasing in duration of storage regardless of the extender used, *viz*. 4 h (71%; n = 89), 28 h (61%; n = 89), 52 h (39%; n = 89); 76 h (28%; n = 90). However, whatever the duration of storage, no seminal plasma (P = 0.23) nor any extender effect (P = 0.60) was recorded on the % kidding after AI (Table 1).

Table 1 Effects of removal of seminal plasma, extender and duration of storage on % kidding after artificial insemination

Seminal plasma	Extender	Duration of storage			
		4 h	28 h	52 h	76 h
SP-	Milk	63.6 (22)	59.1 (22)	52.1 (23)	26.1 (23)
SP+		81.8 (18)	63.6 (22)	34.8 (23)	26.1 (23)
SP-	NPPC-BL	65.2 (23)	56.5 (23)	22.7 (22)	27.3 (22)
SP+		72.7 (22)	63.6 (22)	47.6 (21)	31.8 (22)

(n) - females inseminated

In Experiment 2 the fertility decreased progressively (P < 0.01) with duration of storage: 4 h (69%; n = 78); 28 h (55%; n = 80), 52 h (53%; n = 64), 76 h (38%; n = 93). However, whatever the duration of storage, no dilution rate (P = 0.96) nor any extender effect (P = 0.73) was observed on % of kidding (Table 2).

Table 2 Effects of dilution rate, extender and duration of storage on % kidding after artificial insemination

Dilution rate	Extender	Duration of storage			
		4 h	28 h	52 h	76 h
250 x 10^6 /mL	Milk	63.2 (19)	52.4 (21)	68.8 (16)	39.1(23)
500 x 10^6 /mL		68.4 (19)	57.9 (19)	31.3 (16)	41.7 (24)
250 x 10^6 /mL	NPPC-BL	70.0 (20)	63.2 (19)	56.3 (16)	26.1 (23)
500 x 10^6 /mL		75.0 (20)	47.6 (21)	56.3 (16)	47.8 (23)

(n) - females inseminated

For both experiments a high fertility rate was obtained (± 70%) after four hours of *in vitro* storage. Beyond four hours, a dramatic decrease in kidding rate was observed, reaching ± 40% at 76 hours of storage. This confirmed that the fertilizing capacity of sperm stored in milk lasted only between 12 and 24 h (Dauzier, 1966). Although the protection of sperm cells was greater after *in vitro* storage for seven days in NPPC-BL extender compared with raw milk (Leboeuf *et al*., 2003), the fertility rate decreased in each of the two extenders whatever the duration of storage.

Evidence was given in Experiment 1 that the removal of seminal plasma by centrifugation at collection does not improve fertility rate. This confirms the previous *in vitro* results indicating no detrimental effect of seminal plasma when sperm was stored for seven days at 4 °C, in spite of the presence of residual phospholipides in NPPC (Faulquant *et al*., 1992) and in milk. The activity of the lipase from bulbourethral glands is probably reduced at 4 °C compared to 37 °C (Pellicer-Rubio, & Combarnous, 1998).

The total number of spermatozoa inseminated per female is one of the main factors affecting fertility. Corteel & Leboeuf (1990) have demonstrated that acceptable fertility (around 60%) may be obtained with a total of 100 x 10^6 frozen spermatozoa packed in 0.25 mL straws. Based on the same number of total

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spermatozoa per straw (100 x 10^6), but with a variable inseminated volume of 0.40 mL per straw and 0.20 mL per straw, we have compared two concentrations of spermatozoa, 250 x 10^6 *vs*. 500 x 10^6. The reduction of the concentration of spermatozoa per straw, which corresponded to an increased dilution rate, did not improve the fertility after AI whatever the storage duration. Irrespective of the conditions of storage, the spermatozoa were damaged as the duration of storage increased. The reduction of the sperm integrity may contribute to an accumulation of the toxic products of metabolism. Shannon & Curson (1972) showed that dead sperm produced H_2O_2, which could reduce the membrane integrity and compromise the survival of sperm. These events were accompanied by a decline in transport and survival of spermatozoa in the female reproductive tract and by a reduction of fertility. These phenomena increase with the concentration of sperm and the storage duration. However, under our conditions we did not observe a favourable effect of dilution rate on fertility post storage. It is also possible that the process of liquid storage anticipates the maturation of sperm membrane and the capacitation, which reduces viability and limits the fertile life of sperm (Maxwell & Watson, 1996).

Conclusion

In spite of the modifications of storage conditions tested, the fertility rate decreased when the duration of storage before AI increased from 4 to 76 hours. More research is required to identify the origin of the failures, which may include the fertility potential of sperm, transport of sperm through the genital tract or early embryo loss before or after implantation.

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